Regulation of Fetal and Adult Hemoglobin Formation in Patients with Sickle Cell Disease Transfused to Normal Hematocrits

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FETAL HEMOGLOBIN (Hb F) is the major respiratory pigment formed during intrauterine life. In the adult, however, only trace concentrations normally are present. In man there are at least two distinct conditions in which genetic factors cause elevated levels of Hb F in adult life. They are the hereditary persistence of fetal hemoglobin syndromes and F-thalassemia (or Type II thalassemia).[1,2] It is not known why increased amounts of Hb F are sometimes associated with other clinical states, such as A_2 (or Type I) thalassemia, sickle cell anemia, aplastic anemia, leukemia, or pernicious anemia. Although marrow stress has been considered as a possible factor, there is no relation of the level of Hb F to the degree of anemia. Elevated levels of Hb F may, in fact, persist for some months following remission in aplastic anemia or pernicious anemia.[3,4]

The lack of immediate connection between the degree of anemia and the synthesis of increased quantities of Hb F was experimentally studied by Reed, Bradley, and Ranney in two patients with sickle cell anemia.[5] They found that fetal hemoglobin synthesis persisted in spite of partial relief of the stress of anemia by the chronic administration of blood transfusion. They also found that the relative amount of Hb F in the recipients' red cells increased during the first weeks following the institution of transfusion therapy. They considered that this finding was related solely to the more prolonged survival time of red cells containing relatively greater amounts of hemoglobin F as compared to those containing relatively greater amounts of hemoglobin S (Hb S).[6] They did not rule out the possibility, however, that fetal hemoglobin synthesis may have continued unsuppressed by the transfusions, only that of the adult hemoglobin

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being so affected. The purpose of the study to be described was to investigate this possibility. The synthesis of hemoglobins S and F was assessed in cohorts of reticulocytes emerging into the blood following the acute suppression of erythropoiesis by blood transfusion in three patients with sickle cell disease and elevated levels of Hb F. The formation of both hemoglobins was essentially completely suppressed within a few days of the transfusions, suggesting that they are both acutely regulated by the same control mechanisms and that the increasing relative amount of Hb F seen by Reed and co-workers following transfusion was indeed related solely to the more prolonged survival time in the circulation of erythrocytes containing greater quantities of Hb F.

Materials and Methods

After control observations, two young women with typical homozygous sickle cell anemia (L. B. with 7.6 per cent Hb F and L. G. with 4.8 per cent Hb F) and one middle-aged man with interacting sickle thalassemia (J. G. with 28.2 per cent Hb F and no detectable Hb A) were given 4 units of packed red cells over a 2-day period, rasing their hemoglobin concentration to 14–16 Gm. per 100 ml. The absence of Hb S in the transfused blood was demonstrated by starch gel electrophoresis. Heparinized blood specimens were taken during the period of reticulocyte suppression which followed, and in two patients also during the period of reticulocyte recovery. These fresh specimens were incubated with 10 μc. 1-leucine-U-C^{14} (SA 246 μc. per uM*) per 20 ml. blood, with 1 mg. per ml. added glucose. Incubations of 5 ml. mixture per 50 ml. closed Erlenmeyer flask with air atmosphere were continued for 1 hour (L. B. and J. G.) or 4 hours (L. G.) in a Dubnoff shaker at 37 C. Hemolysates were prepared after washing the red cells at 4 C., nonradioactive carrier leucine was added to diminish possible adsorption of isotope, and the stroma was removed by centrifugation at 10.000 g for 1 hour. The hemoglobins were then separated on columns of Amberlite CG 50.[7] Hemoglobin F was further purified by gradient elution from carboxymethylcellulose,[8] and hemoglobin S was purified by twice-repeated starch granule electrophoresis at pH 8.6. Samples were concentrated with polyethylene glycol and identified by vertical starch gel electrophoresis (tris-EDTA-borate buffer at pH 8.6) and by agar gel electrophoresis (phosphate-citrate buffer at pH 6.45). The C^{14} specific activity of duplicate samples was determined.[9] The duplicate variability was ±9.2 per cent of the mean (95 per cent probability range). The proportions of hemoglobins A, S, and F were determined on all specimens in separate procedures combining the alkali resistance measurement,[10] Amberlite chromatography, and starch granule electrophoresis on small samples in the cyanmethemoglobin form. An expression of total hemoglobin synthesis was obtained by multiplying specific activity by the absolute amount of Hb S or F present per ml. of the incubated specimen.

Results

The results in all three patients indicated that both fetal and adult hemoglobin (in this instance Hb S) production almost completely ceased in the reticulocytes produced four days after the erythropoietic stimulus of anemia was abolished by transfusion to normal hemoglobin levels (Fig. 1). In two of the patients observations were made during the recovery of reticulocytes as the hemoglobin concentration was falling to the original level (Table 1). In both, fetal hemoglobin production returned at approximately the same time as adult hemoglobin production.

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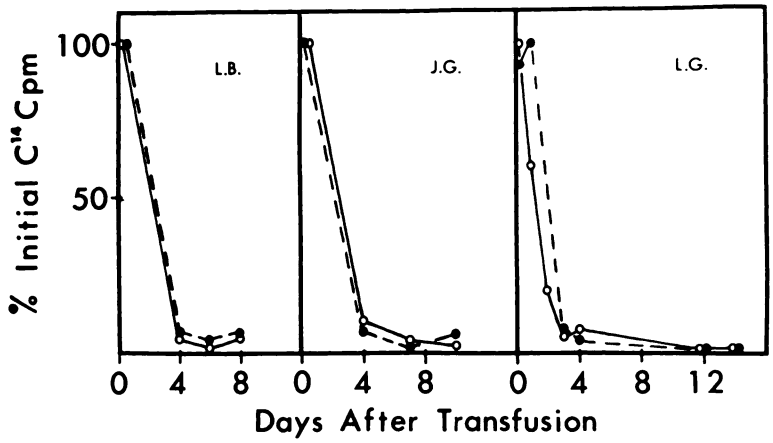

Fig. 1.—Synthesis of hemoglobin S (○——○) and hemoglobin F (● - - - ●) in cohorts of reticulocytes emerging during the post-transfusion period in three patients with sickle cell disease. Results are expressed in terms of per cent of the initial total cpm. (For L. G. Hb F, day 1 was used as the 100 per cent value.)

In one patient (L. B.) bone marrow aspirations taken from the posterior iliac crest were also incubated as described above. The aspirates, diluted somewhat in peripheral blood, were obtained before transfusion and again four days after. Comparison of the simultaneous blood and marrow specimens indicated that the marrow was not a preferential site of Hb F synthesis, since the ratios of Hb F to Hb S specific activity were approximately the same in both sites (Table 2). This study suggests that the reticulocytes are a reasonably good reflection of the events which preceded them in the marrow.

Whether or not the decline and subsequent return of Hb S and Hb F synthesis are exactly parallel cannot be concluded from the available data. While Hb F synthesis in L. G. reticulocytes appeared to increase during the first day following transfusion, this may have been related to experimental error. Likewise, the ratios of total synthesis of the two hemoglobins are difficult to interpret during the period of the suppression, because the low count rates markedly increased the counting error to values as high as 70 per cent (±2 coefficients of variation). (For values outside of the suppression phase the counting error was usually less than 5 per cent.) Thus, in order to obtain a very precise picture of the exact relationships of the synthesis of the two hemoglobins during the four days required to achieve fairly complete suppression of erythropoiesis, more numerous samples would be required, and in order to accurately determine the ratios of the residual synthesis of the two hemoglobins during maximum erythroid suppression, larger samples would be necessary to reduce the counting error sufficiently.

The proportions and absolute amounts of the individual hemoglobins in the peripheral blood during the study period are included in Table 3. As shown by Singer and Fisher in 1952,[6] Hb S disappeared from the circulation more rapidly than Hb F. This relative difference in survival times finds a reflection in the fact that the Hb F proportion of the total endogenous hemoglobin continued

Table 1.—*Incorporation of 1-Leucine-U-C^{14} into Hemoglobins S and F during in Vitro Incubation of Whole Blood Reticulocytes Taken during and after Erythroid Suppression Produced by Blood Transfusion*

Days after Transfusion	Hemoglobin Synthesis (cpm per ml. blood)		Hemoglobin Specific Activity (cpm per mg.)		Whole Blood Hemoglobin Concentration (Gm. per 100 ml.)	Absolute Reticulocyte Count per mm.³
	Hb S	Hb F	Hb S	Hb F		
			*Patient L. B.**			
0	16,400	298	191	42	9.3	31 × 10^4
4	740	20	12	2.8	14.4	13 × 10^4
6	210	10	3.6	1.5	13.0	4.4 × 10^4
8	350	6	6.8	0.9	11.2	4.3 × 10^4
14	61,600	1,520	1,368	230	8.5	34 × 10^4
15	22,000	526	433	85	8.4	33 × 10^4
			Patient J. G.			
0	8,380	1,760	102	55	11.4	27.0 × 10^4
4	850	104	14	3.7	16.1	21.0 × 10^4
7	240	29	3.8	1.2	15.8	6.0 × 10^4
10	190	60	3.3	2.0	15.1	10.7 × 10^4
			Patient L. G.†			
0	104,500	2,850	1,608	864	6.8	45 × 10^4
1	63,400	3,400	1,377	1,173	9.1	39 × 10^4
2	20,200	—	367	—	14.3	33 × 10^4
3	5,830	280	108	70	14.2	19 × 10^4
4	8,520	133	158	35	13.7	12 × 10^4
12	80	00	3.0	0	10.9	0.4 × 10^4
14	00	75	0	25	10.1	0 × 10^4
19	1,290	32	92	12	9.4	4.3 × 10^4
21	4,580	286	327	124	8.9	5.8 × 10^4
22	5,000	218	454	99	8.7	3.2 × 10^4
26	15,480	356	858	155	8.7	9.0 × 10^4
55	16,500	600	551	286	8.2	37.3 × 10^4

*Note that the transfused cells had an abnormally short lifespan in L. B. for unknown reasons.

†Values are corrected for carrier fetal hemoglobin added to L. G. hemolysates in order to improve recovery. Hb F sample of day 2 was accidentally lost.

to rise for several weeks after transfusion. In all three patients the Hb S concentration dropped suddenly by 20 to 30 per cent of its initial value within one day of transfusion. This observation may be indicative of a short-lived population of erythrocytes containing mostly Hb S. A second possibility is that the process of transfusion actually induced an acute sequestration of the recipients' cells. Patient L. B. experienced a hemolytic reaction to the transfused blood associated with a painful crisis. This was presumed to have been caused by an undetected antibody induced by previous transfusion and resulted in an accelerated removal of hemoglobin A from the circulation. A brisk reticulocyte response temporarily accompanied by increased numbers of circulating normoblasts to 700 per mm.³ occurred in response to this rapid return to the anemic state. In patient L. G. the level of Hb F increased by about 0.1 Gm. per 100 ml. after transfusion, suggesting that she received a small but significant quantity

Table 2.—*Comparison of Simultaneous Studies of Peripheral Blood and Bone Marrow of Patient L. B. in which the Incorporation of 1-Leucine-U-C^{14} into Hemoglobins S and F was Measured

Days after Transfusion	Hemoglobin S Specific Activity (cpm. per mg.)		Hemoglobin F Specific Activity (cpm per mg.)		Hb F S.A. / Hb S S.A. Ratio	
	Blood	Marrow	Blood	Marrow	Blood	Marrow
0	191	424	42	89	.22	.21
4	12	53	2.8	9.2	.23	.17

*Marrow nucleated cell counts on day 0 and day 4 were 20,000 per $mm.^3$ and 13,000 per $mm.^3$, with 23 per cent and 15 per cent nucleated red cells, respectively.

of Hb F in the transfused blood. Although this would affect the specific activity determination, it would not influence the value for total synthesis. On all baseline studies, the specific activity of Hb S exceeded that of Hb F, as shown by Karpatkin.[11] This is explained by the more rapid turnover rate of Hb S, as discussed previously. Since steady state conditions are not present following the transfusion, subsequent specific activity measurements cannot be directly interpreted.

Discussion

The finding that suppression of erythropoiesis by means of blood transfusion caused almost complete cessation of formation of both Hb F and Hb S in patients with sickle cell disease suggests that both of these hemoglobins are under similar physiologic control mechanisms. If we assume that the serial cohorts of reticulocytes studied are an accurate reflection of the activity of the marrow normoblasts which preceded them, we may conclude that the slowed rate of differentiation of stem cells into pronormoblasts, coming about as a result of a diminished production of erythropoietin, affects the synthesis of these two hemoglobins about equally. The similarity of the control mechanism was again evident, as the synthesis of both hemoglobins subsequently returned almost simultaneously, indicating that those stem cells which previously were capable of differentiating into fetal hemoglobin-producing erythroblasts retained this capacity during the relatively short time interval of erythroid suppression. Thus, the increasing percentage of Hb F in the endogenous erythrocytes of patients with sickle cell disease following blood transfusion is due exclusively to the more prolonged survival time of the endogenous red cells containing relatively greater amounts of Hb F, as Reed and associates claimed.[5] The same explanation may be held as correct for the observation that fetal hemoglobin levels are higher during aplastic crisis in sickle cell anemia than following recovery.[12] The findings do not support the contention of Necheles and associates that only adult, but not fetal, hemoglobin production is suppressed by transfusion.[13]

Since the fetal and adult hemoglobins are heterogenously distributed among the circulating red cells in the conditions under discussion, it seems likely that a similar circumstance applies to the synthesis of the two hemoglobins within the marrow normoblasts. It is important to know whether peripheral blood

Table 3.—*Proportions of the Individual Hemoglobins in the Peripheral Blood of Three Patients with Sickle Cell Disease following Transfusion of 4 Units of Packed Cells (Hemoglobin A_2 is Included with Hemoglobin S)*

Days after Transfusion	Hemoglobin S		Hemoglobin F			Transfused Hemoglobin A	
	% of Total	Gm. Per 100 ml.	% of Total	Gm. Per 100 ml.	% of Total Endogenous Hb	% of Total	Gm. Per 100 ml.
			Patient L. B.				
0	92.4	8.6	7.6	0.71	7.6	0	0
1	50.2	6.9	5.3	0.73	9.6	44.5	6.1
2	43.9	6.4	4.9	0.72	10.1	51.2	7.5
3	47.7	7.3	4.8	0.74	9.2	47.5	7.3
4	42.8	6.2	5.0	0.72	10.4	52.2	7.5
5	42.1	5.6	5.2	0.69	11.0	52.7	7.0
6	44.1	5.7	5.3	0.69	10.8	50.6	6.6
7	46.3	5.8	5.8	0.72	11.0	47.9	6.0
8	46.4	5.2	6.0	0.67	11.4	47.6	5.3
9	50.6	5.0	6.6	0.65	11.5	42.8	4.2
12	51.0	3.8	6.9	0.51	11.8	42.1	3.1
13	56.8	4.9	8.0	0.70	12.5	35.2	3.1
14	53.5	4.5	7.8	0.66	12.8	38.7	3.3
15	60.8	5.1	7.4	0.62	10.8	31.8	2.7
16	57.1	4.8	7.0	0.59	11.0	35.9	3.0
			Patient J. G.				
0	71.8	8.2	28.2	3.2	28.2	0	0
1	49.7	6.5	20.6	2.7	29.4	29.7	3.9
2	34.4	5.5	17.3	2.8	33.8	48.3	7.8
3	35.1	5.7	17.0	2.8	33.0	47.9	7.8
4	37.8	6.1	17.5	2.8	31.6	44.7	7.2
5	34.3	5.4	17.2	2.7	33.4	48.5	7.7
7	39.7	6.3	15.2	2.4	27.6	45.1	7.1
8	37.8	5.8	17.9	2.8	32.6	44.3	6.9
10	38.3	5.8	20.0	3.0	34.2	41.7	6.3
15	32.3	4.4	19.2	2.6	37.2	48.5	6.5
18	34.9	4.6	19.2	2.5	35.3	45.9	6.1
23	35.3	4.9	19.1	2.6	34.7	45.6	6.3
30	35.4	4.6	19.1	2.5	35.2	45.4	6.0
37	33.4	3.6	18.2	2.0	35.8	48.4	5.2
44	45.0	5.0	19.4	2.2	30.6	35.6	4.0
			Patient L. G.				
0	95.2	6.5	4.8	0.33	4.8	0	0
1	50.8	4.6	3.2	0.29	5.9	46.0	4.2
2	38.3	5.5	3.0	0.43	7.3	58.7	8.4
3	38.4	5.4	2.8	0.40	6.9	58.8	8.3
4	39.4	5.4	2.8	0.38	6.6	57.8	7.9
5	38.4	5.0	2.7	0.35	6.6	58.9	7.7
6	34.4	4.4	2.8	0.36	7.5	62.8	8.0
8	29.6	3.6	2.8	0.34	8.6	67.6	8.2
9	27.4	3.3	3.0	0.37	10.1	69.6	8.5
12	23.6	2.6	2.8	0.30	10.3	73.6	8.0
14	23.9	2.4	3.0	0.30	11.1	73.1	7.4
19	15.4	1.4	2.9	0.27	16.2	81.7	7.7
21	15.2	1.4	2.6	0.23	14.1	82.2	7.3
22	12.7	1.1	2.5	0.22	16.7	84.8	7.4
26	20.2	1.8	2.6	0.23	11.3	77.2	6.7
55	36.5	3.0	2.6	0.21	6.5	60.9	5.0

reticulocytes, which represent the end of the erythroid maturation process, are representative of the relative relationships of the formation of the two hemoglobins within the marrow. Evidence has been reported, using isotopic isoleucine as a fetal hemoglobin label, that fetal hemoglobin is preferably made in younger normoblasts.[14] However, studies in one patient in the present investigation suggested that the relationships between Hb S and Hb F synthesis in peripheral blood reticulocytes were similar to marrow samples. Also, in previous in vivo studies the appearance in the peripheral blood of C^{14}-labeled glycine was measured in both fetal and adult hemoglobin in patients with thalassemia. Observations over the first few days after injection of the isotope, a period which forms a mirror image of events during maturation in the marrow, failed to indicate that maximal synthesis of fetal hemoglobin occurred earlier than that of adult hemoglobin.[8] However, thalassemia may not be comparable to sickle cell anemia in this particular. Additional data are required to more clearly delineate the relative timing of fetal and adult hemoglobin formation during the maturation process in the marrow.

Although fetal and adult hemoglobin formation thus appear to be regulated by similar control mechanisms during alterations of erythropoiesis extending over a period of several weeks, it is possible that over a more prolonged period of alleviation of the stimulus of anemia, fetal hemoglobin synthesis may begin to diminish, as suggested by Reed and associates.[5] Thus, a clear distinction must be drawn between acute and chronic alteration in the state of erythropoiesis in considering the factors which regulate the persisting synthesis of Hb F into adult life.

Summary

Two patients with sickle cell anemia and one patient with sickle thalassemia were acutely transfused to normal hemoglobin levels. The synthesis of fetal and adult (in this instance, Hb S) hemoglobins was assessed in cohorts of reticulocytes emerging during the period of erythroid suppression which followed. In two patients observations were also made during the period of recovery from the suppression. Hemoglobin synthesis in the reticulocytes was assessed by means of in vitro incorporation of C^{14}-labeled leucine into the separate hemoglobins. The results in all three patients suggested that both fetal and adult hemoglobin synthesis are under similar physiologic control mechanisms during short-term alterations in the state of erythropoiesis; the formation of both was almost completely stopped soon after transfusion, and both were almost simultaneously reactivated as the patients returned to the anemic state.

Summario in Interlingua

Duo patientes con anemia a cellulas falciforme e un patiente con thalassemia falciforme recipeva acute transfusiones usque al establimento de normal nivellos de hemoglobina. Le synthese de hemoglobina fetal e de hemoglobina adulte (in iste caso, de hemoglobina S) esseva evalutate in cohortes o reticulocytos emergente durante le periodo de suppression erythroide le qual sequeva. In duo patientes le observationes esseva continuate durante le periodo de restab-

limento ab le suppression. Le synthese de hemoglobina in le reticulocytos esseva evalutate per medio del incorporation in vitro de leucina marcate con C^{14} ad in le hemoglobinas separate. Le resultatos in omne le tres patientes suggestionava que le synthese de hemoglobina fetal e etiam de hemoglobina adulte progrede sub simile mechanismos de regulation physiologic durante breve alterationes in le stato del erythropoiese. Le formation de ambes esseva arrestate quasi completemente tosto post le transfusion, e ambes esseva reactivate quasi simultaneemente quando le patientes retornava a lor stato anemic.

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